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PRINCIPAL INVESTIGATOR: Scott H. Frey, Ed.M., Ph.D.

CONTRACTING ORGANIZATION: University of Missouri
St Louis, MO 63130

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14. ABSTRACT In Year 2, we made significant progress on data collection in our multi-day protocol. Our preliminary findings continue to indicate that: 1) Hand transplant recipients utilize the former cortical sensorimotor hand territory when using the affected hand. 2) They continue to exhibit strong evidence for persistent, amputation-related, cortical reorganization. These persistent changes appear to diminish with recovery of hand function, suggesting that cortical reorganization is an important target for post-transplant rehabilitation. 3) Transplant and replant recipients show increased engagement of the parieto-frontal cortical networks involved in visually-guided grasping of objects, and these patterns appear related to recovery of functional hand use. 4) Transplant and replant recipients exhibit a remarkable ability to localize touch on their affected hand in the absence of vision. This level of recovery is difficult to understand given prevailing models in neuroscience that emphasize reinnervation errors. These results are summarized below.					
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1. Introduction

Hand transplant recipients provide a unique opportunity to investigate whether the central reorganizational changes that follow amputation are reversed when afferent and efferent signals between hand and brain are restored, and whether these changes are related to recovery of hand function. Our preliminary functional magnetic resonance imaging (fMRI) data suggest that areas of the sensory and motor cortex devoted to representing the hand prior to amputation come to represent the transplanted hand, even when received decades after amputation. We also find evidence that changes in cortical organization associated with unilateral hand amputation may not be fully reversed even a decade after transplantation; transplant recipients, like amputees, show increased activity in the former hand territory during movements of the adjacently represented face and of the intact hand. Importantly, the level of functional recovery appears to be associated with greater activity in the former hand territory when using the transplanted hand and with less evidence of persistent cortical reorganization. On the basis of our preliminary data, we hypothesize that experience-dependent central (brain) changes play a key role in the functional improvements known to continue throughout at least the first decade following hand transplantation, long after the expected completion of peripheral nerve regeneration. Developing a more complete understanding of the relationship between experience-dependent changes in brain organization and functional outcomes is critical to the long-term success of composite tissue transplantation. This project will yield new insights of fundamental relevance to improving the care and rehabilitation of transplant recipients, and broadly to other patients suffering from peripheral nerve or spinal cord injuries.

2. Keywords

Hand transplant, hand replant, amputation, brain reorganization, sensory, motor, hand function, recovery, functional magnetic resonance imaging

3. Accomplishments

Dr. Frey's laboratory relocated to Washington University School of Medicine in St. Louis on July 1, 2015. Due to unforeseen circumstances, the laboratory moved back to the University of Missouri in Columbia on September 1, 2016.

Prior to our relocation, we made a concerted effort to complete a large portion of data collection at the University of Missouri.

While at Washington University, the lab's efforts were concentrated on quality assurance, preprocessing and analysis of data, manuscript preparation and disseminating results of the project to the scientific community. Several conference presentations were given during this time. We also have submitted a manuscript on this work and several more are in preparation.

Currently, we are awaiting IRB approval at the University of Missouri and from the DoD HRPO before commencing with the completion of data analysis.

As mentioned in recent quarterly reports, our plan is to conduct the remainder of pre- and post-transplant data collection in Louisville at the Christine M. Kleinert Institute (CMKI). This will allow us to evaluate all pending and current transplant patients during their annual visits. The opportunity to perform functional and structural MRI testing in Louisville is possible now because of the recent establishment of a research MRI facility at the adjacent University of Louisville. This will enable our research team to travel to our Louisville several times annually to test the transplant recipients during their mandatory annual checkups, thereby greatly increasing participation. This will involve transporting members of Frey's research team and several pieces of research equipment to Louisville by rental truck and spending time on- to unpack, assemble and prepare equipment, conduct testing and dismantle and pack equipment.

Additionally, the CMKI is an easy 15-minute drive from the Louisville International Airport. This convenient location will increase the likelihood that Johns Hopkins University hand transplant referrals will be interested in traveling to participate (compared to Columbia, Missouri, which is located two hours away from the closest

international airport). We plan to seek data sharing agreements with each institution and collaborate with both teams on manuscript preparation.

Major Goals Achieved: Year Two

Preliminary Data

A. Mapping sensorimotor reorganization in the brains of hand transplant and hand replant recipients.

fMRI sensorimotor mapping protocol. Participants were positioned supine in the scanner and moved different body parts according to auditory cues: “left hand”, “right hand”, “left foot”, “right foot”, or “lips”. Each cue was followed by a series of tones presented at a rate of 1 Hz, and participants were instructed to move the cued body part in pace with the onset of each tone. Movement blocks ended with the cue “stop”, and were 13s in duration, excluding movement instruction and stop cues. Each movement block was followed by 18s rest periods. Participants were asked to keep their eyes closed throughout.

Evidence for the reversal of amputation-related changes in cortical primary sensorimotor map organization. DR is a right-hand dominant male who suffered traumatic amputation of his left hand proximal to the wrist at the age 23 years. Thirteen years after amputation, patient DR underwent successful allogeneic hand transplantation. The data presented here were collected at 15 (Session 1) and 26 (Session 2) months post-transplant. During this interval of time, DR’s hand function improved substantially. Carroll Test from 67 to 86; Dash test from 24.14 to 8.6.

The fMRI results indicate marked reduction in the extent to which DR activates his ipsilateral sensorimotor cortex when moving his *non-transplanted hand* between testing session 1 & 2. This pattern reflects functional reorganization following the amputation that appear to persist even after the transplant (**Figure 1**). In patient DR, movements of his transplanted hand robustly activated contralateral sensorimotor cortex (SMC) and ipsilateral cerebellum, and the magnitude, location, and extent of this activity was remarkably consistent across Sessions 1 and 2. At 15 months post-transplant, movements of DR’s non-transplanted hand also resulted in robust ipsilateral SMC activity, closely overlapping with preferential responses evoked by movements of his transplanted hand. *In other words, a considerable portion of DR’s functionally-defined sensorimotor hand area contralateral to – and presumably devoted to the control of – his transplanted hand was also activated by movements of his non-transplanted hand.* Most exciting, the results from Session 2, at 26 months post-transplantation, reveal a striking reduction in the magnitude and extent of ipsilateral SMC activity during movements of DR’s non-transplanted hand, and these changes are within the area of cortex preferentially responsive to movements of his transplanted hand. As this area of cortex continues to selectively respond to movements of DR’s transplanted hand, paralleled by continuous improvements in sensory and motor function, response sensitivity to movements of the non-transplanted hand is reduced. As illustrated in **Figure 2**, these patterns differ from those exhibited by individual controls. Further, as is evident in **Figure 3**, we see very stable responses during hand movements in controls across two testing sessions, which suggests that the longitudinal changes in DR are valid and reliable markers of experience-dependent plasticity.

A second transplant – EH – also shows evidence of amputee-like bilateral SMC activity when moving his (left) non-transplanted hand. EH was tested 65 mths post transplant when his hand was functioning at a moderate level (Dash score = 53, Carroll score = 76). He was an amputee for 32 mths before receiving a transplant proximal to the wrist of his right dominant hand (**Figure 4**).

We find a different pattern in patient WH, who experienced an distal forearm amputation and immediate replantation. WH Following replantation of his amputated left hand, WH shows a different pattern than the transplant recipients. He exhibits very significant bilateral activation when moving either the affected or the unaffected hands (**Figure 5**).

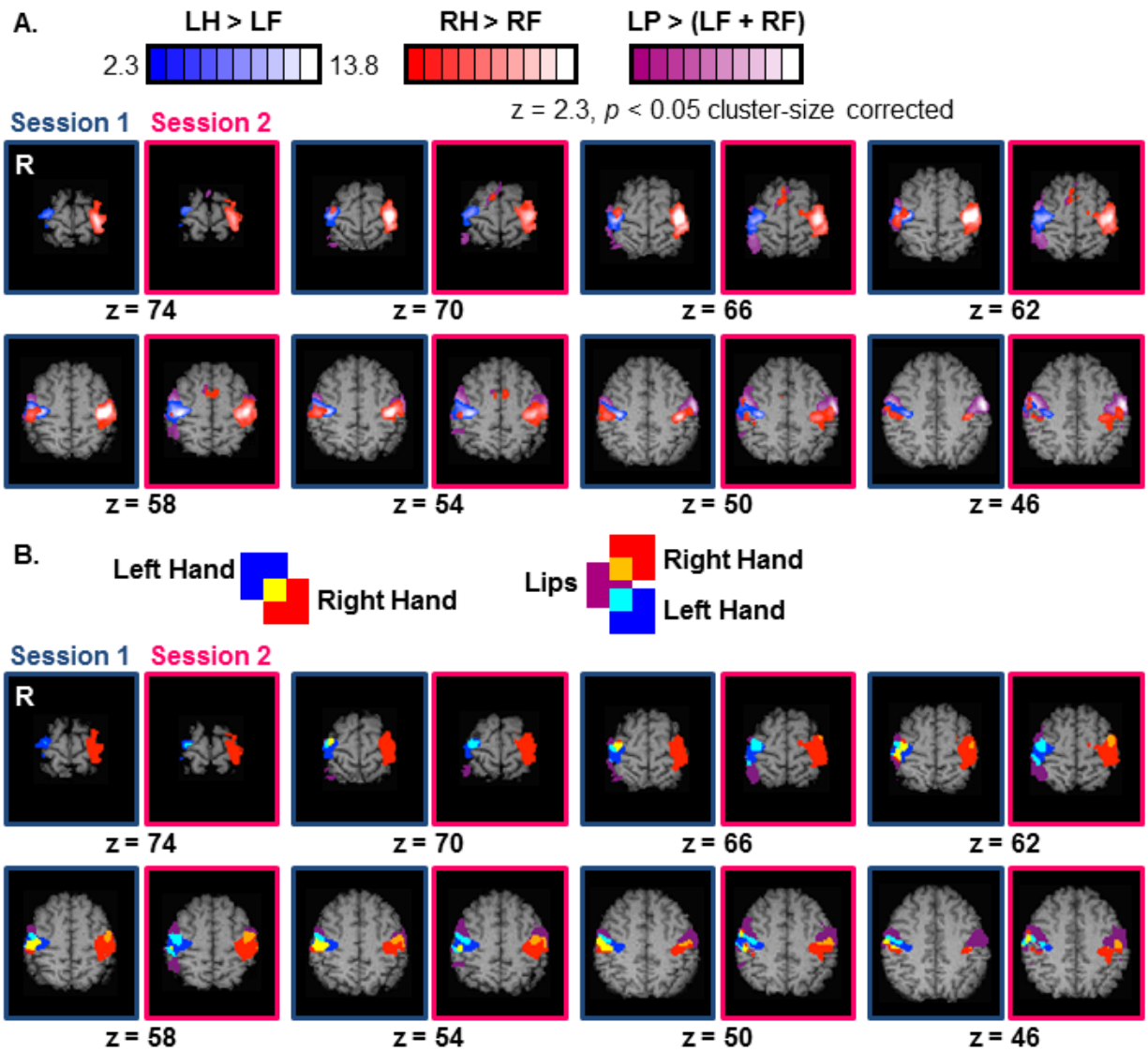


Figure 1. Patient DR: somatomotor map organization over time. (A) Activity maps showing preferential responses for movements of the left hand (blue-to-white), right hand (red-to-white), and lips (purple-to-white) in patient DR at 15 (Session 1) and 26 (Session 1) months post-transplant. Axial slice images from Session 1 (blue outline) and Session 2 (pink outline) are shown side-by-side to facilitate comparisons. Set to the same thresholds, the maps indicate the statistical strengths of the effects for each of the three independent contrasts, as well as the locations of peak maxima. (B) The same activity maps shown in (A) masked as solid-colored-voxels to better illustrate the voxel-wise spatial extents and points of overlap between maps. Most notably, there is a striking reduction in the extent of activity preferentially responsive to movements of DR's right, non-transplanted hand within his right (ipsilateral) sensorimotor cortex from Sessions 1 to 2 (compare yellow-colored voxels, slices $z = 58$ through 46). LH = left hand; RH = right hand; LP = lips; LF = left foot; RF = right foot.

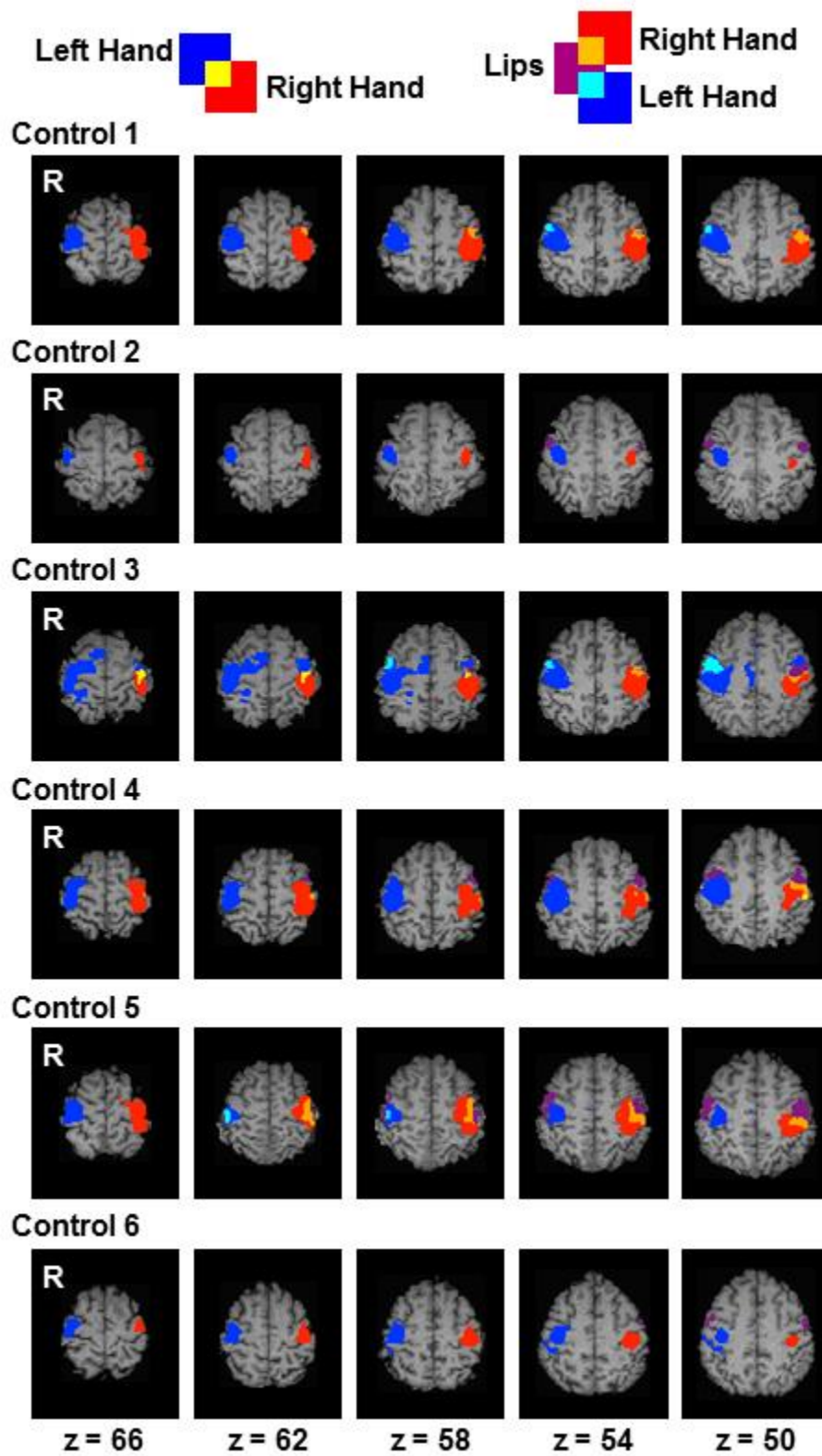


Figure 2. Data from 6 healthy, age- gender- and handedness-matched controls. Note the absence of areas exhibiting increased activity for movements of both hands.

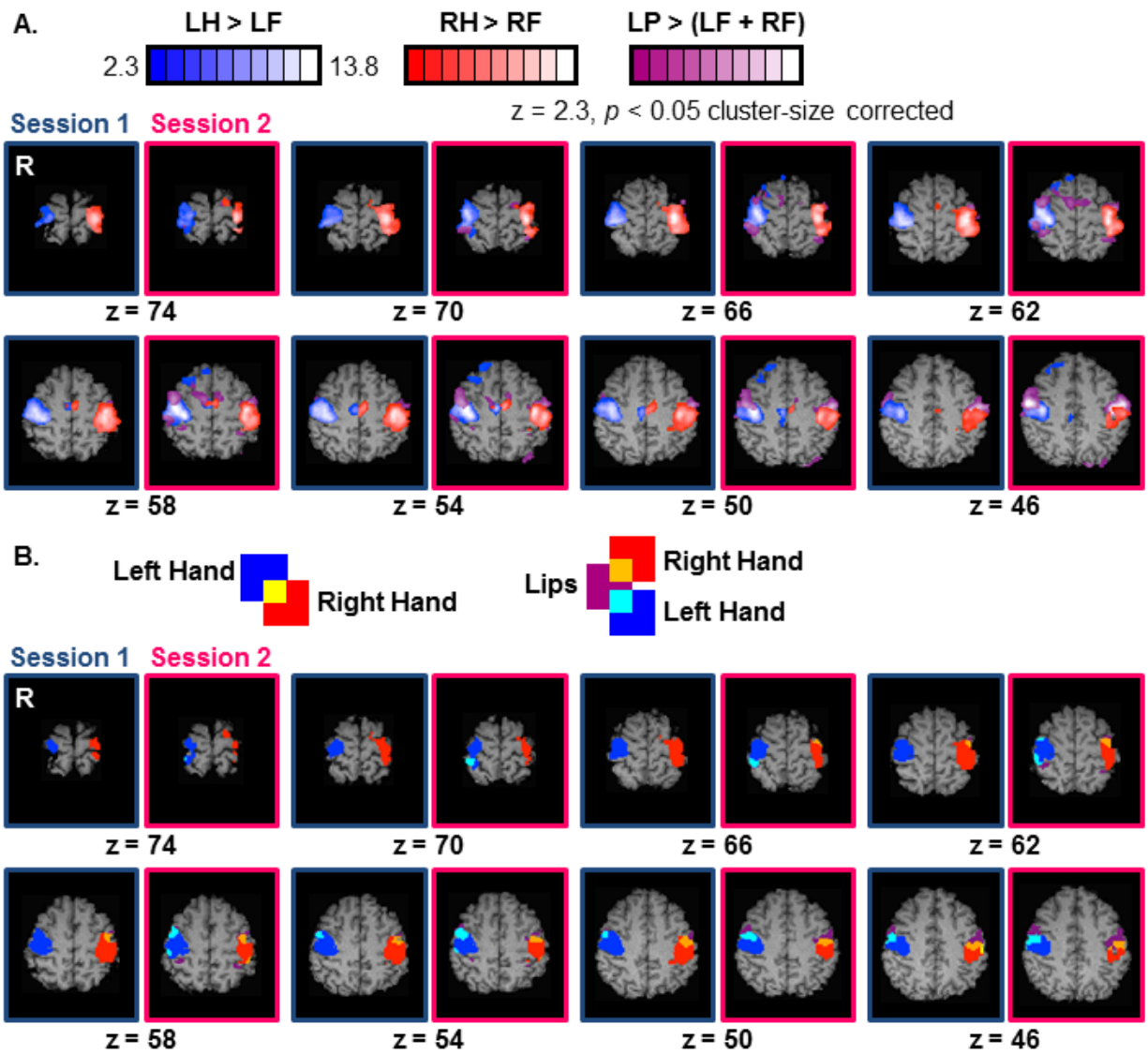


Figure 3. Example Control participant (C1): somatomotor map organization over time. Compare with Figure 1. A) Unlike patient DR, results from an age-, gender-, and handedness-matched healthy control participant show little evidence of ipsilateral sensorimotor cortex activity for movements of either hand, for either session. B) Specifically, in Session 1 only, a total of 58 voxels were activated within in left sensorimotor cortex for movements of the left (ipsilateral) hand, 37 of which overlapped with responses for movements of the right (contralateral) hand (evident as yellow-colored voxels in slice $z = 46$). LH = left hand; RH = right hand; LP = lips; LF = left foot; RF = right foot.

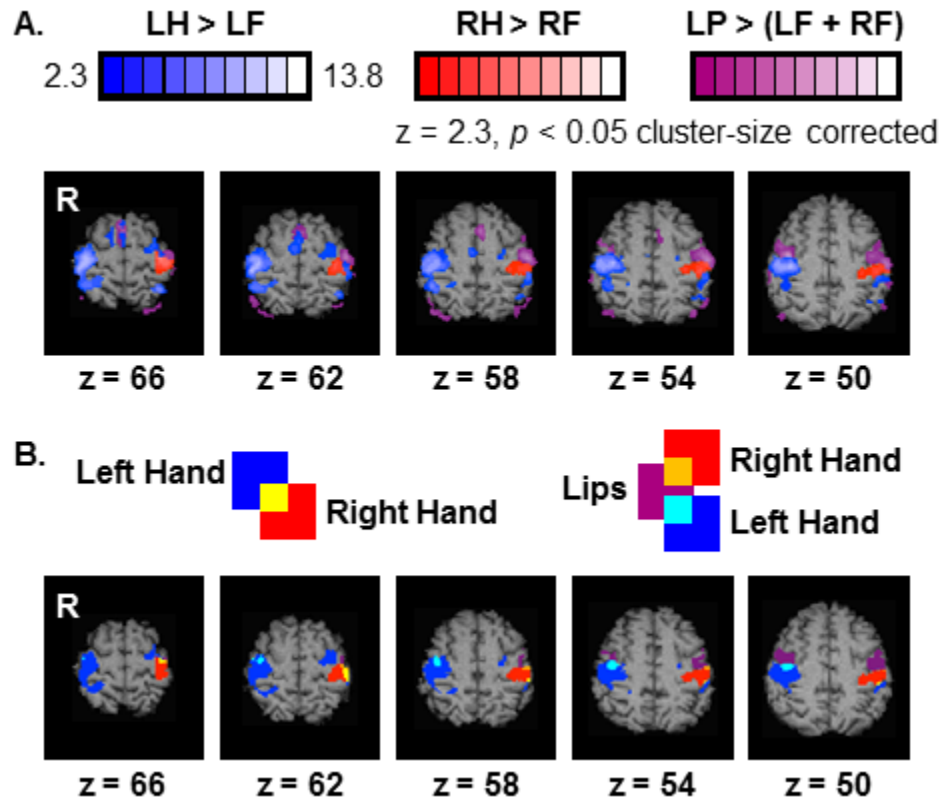


Figure 4. Data from right hand transplant patient EH. As for DR (Fig. 1), we see increased bilateral activity during movements of the intact hand.

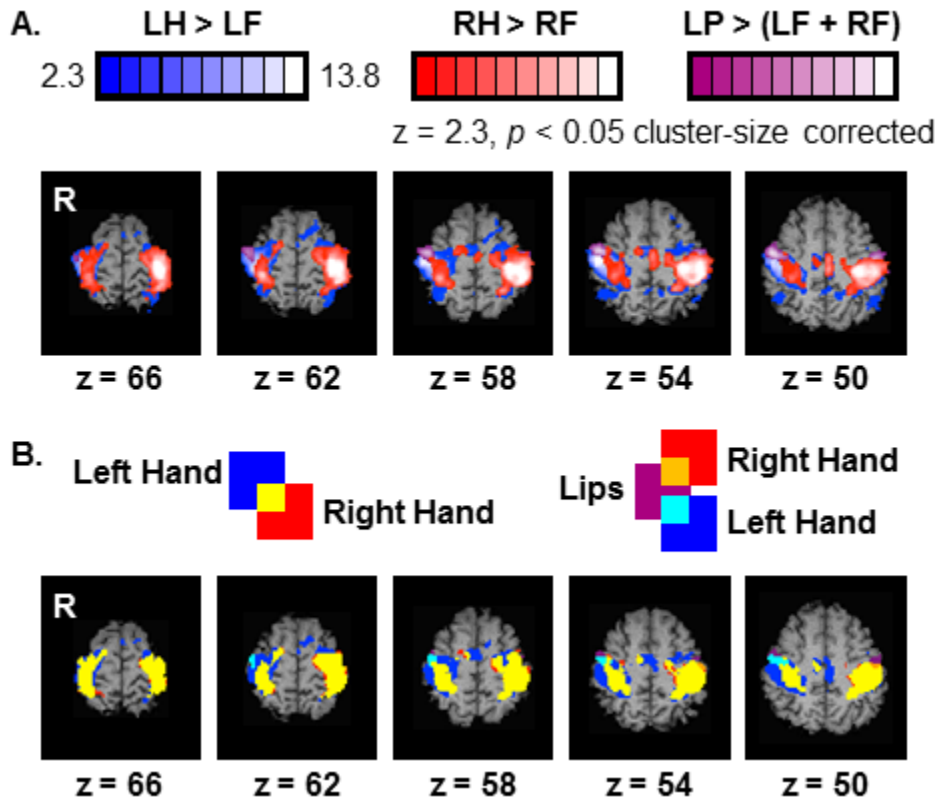
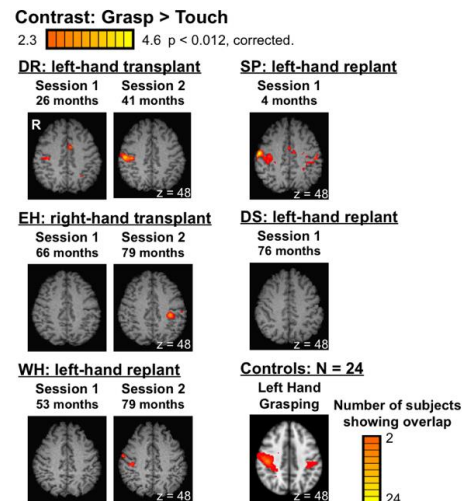


Figure 5. Data from hand replant recipient patient WH. Following replantation of his amputated left hand, WH shows a different pattern than the transplant recipients. He exhibits very significant bilateral activation when moving either the affected or the unaffected hands.

Figure 6: Grasp-selective activity in patients and controls. Activation maps shown per patient, per session are set to reliable statistical thresholds, and the data shown for controls reflects the number of subjects showing active voxels at these same single-subject level thresholds. In at least one testing session, nearly all (4/5) patients show significant activity for grasping within contralateral AIPC, similar to that seen in the majority (19/24) of controls. At reduced thresholds, (not shown here), this activity was also evident for patients EH and WH, Session 1, and DS, as well as for 3/5 of the controls that also failed to show significant effects at corrected levels. Patients DR, SP, and DS (at reduced thresholds) also showed evidence of ipsilateral AIPC activity, similar to many (7/24 corrected; 15/24 uncorrected) controls.



Neural Control of Reaching and Grasping with a Transplanted or Replanted Hand

We have collected data from five patients, two hand transplant and three replant cases, and 24 age-, gender-, and handedness-matched healthy control participants. One replant, and two transplant patients, and one control participant completed the paradigm twice, approximately one year apart. Although there is considerably variability across patients, grasping with the re/transplanted hand tends to activate contralateral anterior intraparietal cortex (AIPC) (**Figure 6**). This pattern of brain activity is qualitatively similar that that observed in

healthy controls, and suggests that the functional organization of brain areas dedicated to the control of grasping can be reestablished after traumatic hand amputation and surgical re/transplantation. In all three patients tested longitudinally, the statistical strength and extent of grasp-selective activity in contralateral AIPC increased over time (Figure 1). This suggests recovery associated with regaining stronger representation (more neural tissue) in the former cortical hand territory

Sensory Localization: Behavioral Consequences of Central Reorganization

We have been working on cleaning and analyzing locognosia data from both the amputee participants and the transplant/replant participants. Figure X is the latest data showing a clear improvement in the ability to localize touch on the palm of the surgically attached hand in transplant patients over time. We have been working on cleaning and analyzing locognosia data from both the amputee participants and the transplant/replant participants. Figure 1 is the latest data showing a clear improvement in the ability to localize touch on the palm of the surgically attached hand in transplant patients DR and EH over time. Transplant recipients GF and MS were only tested at a single time point, 8 and 10 years after their transplants respectively. Patients CH, JS, PP, and RW are hand replant recipients.

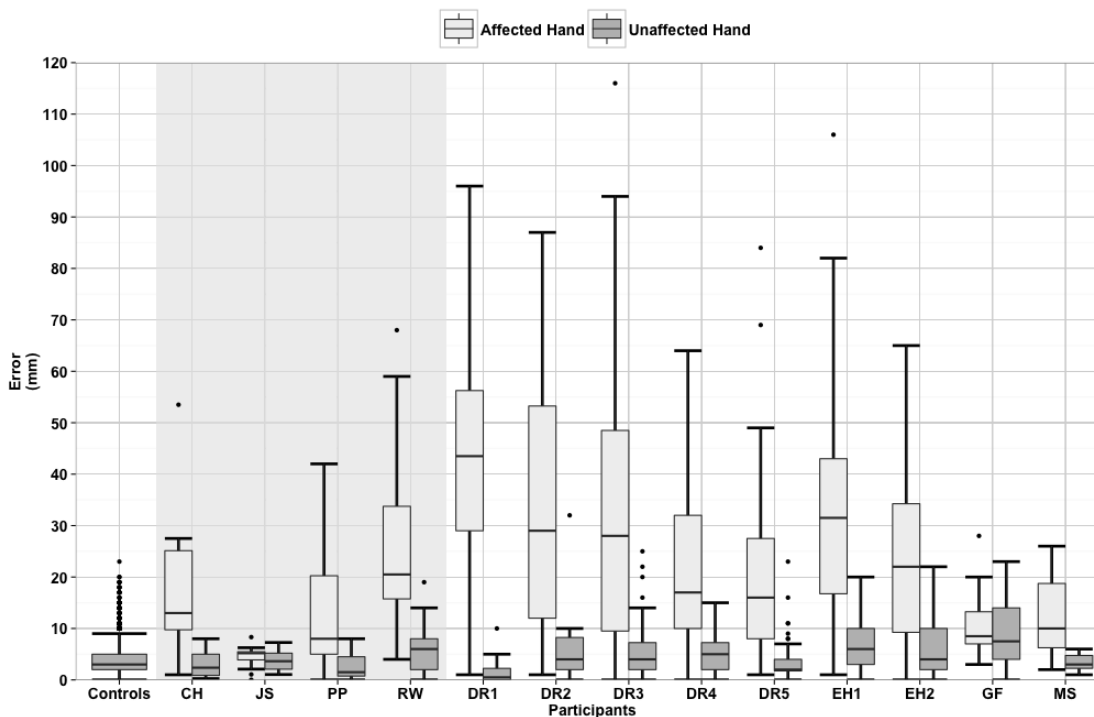


Figure 7 illustrates the accuracy with which our hand replant (gray shaded region) and hand transplant recipients are capable of localizing a light touch on the affected vs. unaffected hand. Note that DR has been evaluated on three different occasions. Overall, both groups exhibit lower mean error rates that are related to the time post-surgery. Transplant recipients on the far left show remarkably accurate recovery when tested 8 (GF) and 10 (MS) years post-transplant. This is consistent with evidence indicating continued gradual improvements in hand function long after the peripheral nervous system has completed reinnervation. We hypothesize that these changes are attributable to central (brain) adaptations.

4. Impact

It is too early to discern many of the broader impacts of this work. At this stage, we can say that the trainees involved in the project have benefited significantly in terms of their skills in this clinically-relevant area of research.

Training Opportunities

This project provided opportunities for postdoctoral and predoctoral trainees to gain additional experience in this area of research.

Postdoctoral fellows: Dr. Kenneth Valyear, Dr. Benjamin Philip
Research Faculty: Dr. Huiling Peng, Dr. Carmen Cirstea
Graduate Students: Nathan Baune, Pin-Wei Chen

5. Challenges/Problems

Moving to the University of Missouri will cause a slight delay while we wait for regulatory approvals.

6. Products

Peer-reviewed Scientific Journals

Valyear, K.F., Philip, B.A., Mattos, D., Kaufman, C., & Frey, S.H. (submitted). “*Grasping with a new hand: Increased engagement of the anterior intraparietal cortex parallels recovery of visually-guided grasping in a former chronic amputee.*”

Philip, B.A., Valyear, K.F., Kaufman, C., Kutz, J., & **Frey, S.H.** (in preparation). “*Experience-dependent functional reorganization in the human sensory cortex and behavioral implications: insights from current and former hand amputees.*”

Valyear, K.F., Philip, B.A., Kaufman, C., Kutz, J., & **Frey, S.H.** (in preparation). “*Experience-dependent functional reorganization in the human sensory cortex and behavioral implications: insights from current and former hand amputees.*”

Mattos, D., & Frey, S.H. (in preparation). “*The control of reaching and grasping with a transplanted or replanted hand: bilateral kinematic effects of unilateral injuries.*”

Baune, N.A., Philip, B.A., & **Frey, S.H.** (in preparation). “*Localization of touch by current and former amputees: is sensory function really related to reorganizational changes in cortical representations?*”

Valyear, K.F., Philip, B.A., Kaufman, C., Kutz, J., & **Frey, S.H.** (in preparation). “*Reconsidering the functional implications of local and distributed reorganization in sensorimotor cortex following hand loss and restoration.*”

Book Chapter

Frey, S.H. (2015). *Why Brain Science is Essential to the Success of Hand Allotransplantation.* In *The Science of Reconstructive Transplantation.* Gerard Brandacher (Ed.). Springer.

Presentations at Professional Conferences (Denotes invited address)*

Frey, S.H. (November, 2015). Cortical organization and recovery of hand functions: lessons from former amputees. First Annual Symposium: Centre Provincial D’expertise Pour les Personnes Victimes De Traumatisme Par Amputation ou Necessitant une Revascularisation Microchirurgicale. Montreal.

Baune N.A., Philip B.A., Kaufman C., Kutz J. & **Frey S.H.** (November 2014). Improved sensation in a replanted or transplanted hand. Poster to be presented at the annual meeting of the American Society of Neurorehabilitation, Washington, D.C.

Baune N.A., Philip B.A., Kaufman C., Kutz J. & **Frey S.H.** (November 2014). Localization of touch on a replanted or transplanted hand: evidence for late improvements that may reflect central adaptations. Poster to be presented at the annual meeting of the Society for Neuroscience, Washington, D.C.

Cirstea M.C., Peng H., & **Frey S.H.** (November 2014). Spectroscopic evidence for lower neuronal metabolism and time-dependent increases in inhibition within the former sensorimotor hand territory of chronic unilateral amputees. Poster to be presented at the annual meetings of the American Society of Neurorehabilitation and Society for Neuroscience, Washington, D.C.

Peng H., Cirstea M.C., Valyear K.F., & **Frey S.H.** (November 2014). Diffusion tensor tractography study of sensorimotor pathways in unilateral hand amputees. Poster to be presented at the annual meetings of the American Society of Neurorehabilitation and Society for Neuroscience, Washington, D.C..

Philip, B.A., & **Frey S.H.** (November 2014). Stability of precision drawing skills acquired with the non-dominant hand and associated changes in functional connectivity between sensorimotor hand representations. Poster presented at the annual meeting of the Society for Neuroscience, Washington D.C.

Philip, B.A., & **Frey S.H.** (November 2014). Skill acquisition with the non-dominant hand and associated changes in functional connectivity between sensorimotor hand representations. Poster presented at the annual meeting of the Society for Neurorehabilitation, Washington D.C.

Valyear, K.F., Philip, B.A., Kaufman, C., Kutz, J., & **Frey, S.H.** (November 2014). Amputation-related changes in inter-hemispheric interactions are reversible through transplantation of the human hand. Poster to be presented at the annual meeting of the Society for Neuroscience, Washington, DC.

Valyear, K.F., Philip, B.A., Kaufman, C., Kutz, J., & **Frey, S.H.** (January, 2015). Cortical reorganization and recovery of limb function following allogeneic hand transplantation. Presented at Extremity War Injuries X: Advancing Health and Function. Washington, DC.

Valyear, K.F., Philip, B.A., Kaufman, C., Kutz, J., & **Frey, S.H.** (May, 2015). Cortical reorganization and recovery of limb function following allogeneic hand transplantation. Presented at the Concept, Objects and Actions Meeting in Rovereto, Italy.

Philip B.A., Valyear K.F., Frey S.H. (November, 2015). Evidence for interhemispheric reorganization in sensory cortex following unilateral upper extremity amputation in humans. Society for Neuroscience, Chicago IL

Philip B.A., Valyear K.F., Frey S.H. (November, 2015). Evidence for interhemispheric reorganization in sensory cortex following unilateral upper extremity amputation in humans. American Society for Neurorehabilitation, Chicago, IL.

Frey, S.H. (April, 2016). "Functional relevance of reorganization in sensory cortex: insights from current and former hand amputees." Annual meeting of the Society for the Neural Control of Movement. Montego Bay, Jamaica.

Philip B.A., Baune N.A., Valyear K.F., Frey S.H. (May, 2016) Interhemispheric reorganization in sensory cortex without functional change following unilateral upper extremity amputation in humans. 9th World Congress for Neurorehabilitation, Philadelphia PA.

Frey, S.H. (May, 2016). *“Lessons on experience-dependent changes in interhemispheric interactions from current and former amputees.”* 9th World Congress for Neurorehabilitation. Philadelphia.

7. Other Collaborating Institutions

This project has been a collaborative effort between Dr. Frey’s team and Dr. Christina Kaufman, collaborating Principal Investigator at the CMKI. Going forward, we will be relocating and her staff are working with Dr. Frey’s team at University of Missouri to obtain an IRB reliance agreement that will allow Dr. Frey’s team to conduct the research protocol at the CMKI. Our teams will work together to submit this modification to the DoD HRPO.